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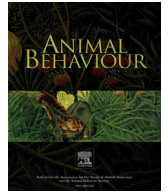
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## How king penguins advertise their sexual maturity

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Reproductive success can improve with experience, which increases with age in many long-lived species. Signals that provide reliable information about age are therefore of importance for mate choice and consequently are under sexual selection. In birds, these are often vocal signals as well as visual signals in the form of plumage coloration. King penguins, *Aptenodytes patagonicus*, are sexually monomorphically ornamented seabirds that perform a complex visual and acoustic courtship display. Coloured beak spots and ear patches contain information about the condition and physiological status of adult males and females, but their role as a signal of age has previously only been studied in young birds. Vocalizations have mainly been studied as signals of individuality and not in the context of courtship. We investigated two multicomponent signals in the context of mate choice by analysing beak spot, ear patch and call parameters of wild king penguins. We explored the relation between these signals and age as well as age classes (chicks, juveniles, adults). Ornament parameters were weakly correlated with in males, but not in females, while acoustic parameters were highly correlated with age in both sexes. The calls' fundamental frequency and energy parameters and all the beak spot parameters reliably classified individuals into their age class. Since age class was redundantly encoded in both acoustic and colour parameters, we hypothesize that calls and ornaments function as back-up signals that increase the chance of accurately conveying the age class of the sender to receivers. King penguins might sequentially analyse age class signals during courtship, where acoustic signals serve as long-range communication when sender and receiver are out of sight, and ornamentation signals become important at close range. We show the importance of considering bimodal, multicomponent signals when studying complex behaviour and discuss how signalling environment, the species' life history and mating system influence the evolution of communication signals.

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A strong influence of age and breeding experience on reproductive success has been observed in several long-lived birds, such as wandering albatrosses, *Diomedea exulans* (Weimerskirch, 1992), short-tailed shearwaters, *Puffinus tenuirostris* (Bradley, Wooller, & Skira, 1995), common terns, *Sterna hirundo* (Nisbet, Winchell, & Heise, 1984) and snow petrels, *Pagodroma nivea* (Angelier, Moe,

Weimerskirch, & Chastel, 2007). Breeding success is related to individual age which has been linked to better territory and antipredator defence (Catty & Furness, 1999), higher foraging efficiency (Le Vaillant et al., 2012, 2013; Zimmer, Ropert-Coudert, Kato, Ancel, & Chiaradia, 2011) and optimized synchronization between partners during incubation and chick rearing (Hatch, 1990). If age has an influence on breeding success, we expect honest signals that provide information about an individual's age, and that are favoured by mutual intersexual selection, to evolve (Jouventin, Lequette, & Dobson, 1999).

Most studies showing that visual and acoustic signals indicate levels of maturity to conspecifics have focused on passerine birds

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(Boves et al., 2014; De Kort, Eldermire, Valderrama, Botero, & Vehrencamp, 2009; Komdeur, Oorebeek, Van Overveld, & Cuthill, 2005; Nemeth, Kempnaers, Matessi, & Brumm, 2012; Rivera-Gutierrez, Pinxten, & Eens, 2010; Siefferman, Hill, & Dobson, 2005; Zipple, Nowicki, Searcy, & Peters, 2019; Zipple, Peters, Searcy, & Nowicki, 2020). Budden and Dickinson (2009) for example showed for western bluebirds, *Sialia mexicana*, that older males had both significantly brighter head plumage and a higher proportion of blue cover on the head and cheek than younger males. Similar evidence for age-related signals in nonpasserine bird species is scarcer but also exists. During the mating season, male frigate birds, *Fregata magnificens*, display their inflatable, red gular pouch and produce simultaneous drumming sounds when females approach. The fundamental frequency of these acoustic courtship signals decreases with increasing age class; plumage iridescence and the presence/absence of white feathers also indicate age class (young, intermediate, old) in males (Madsen, Balsby, Dabelsteen, & Osorno, 2004; Madsen, Dabelsteen, Osorio, & Osorno, 2007).

Massaro, Davis, and Darby (2003) found that age and eye colour were associated with breeding success in yellow-eyed penguins, *Megadyptes antipodes*.

The multicomponent signalling of yellow-eyed penguins and the bimodal signalling of frigatebirds agree with an increasing number of behavioural studies that acknowledge the existence of a complex framework of signals (Candolin, 2003; Hebets & Papaj, 2005; Higham & Hebets, 2013). Indeed, multicomponent and multimodal signalling appear to be the norm rather than the exception (Partan, 2013). First, signals of interest can be described according to their components, for example colourful ornament signals can be characterized by their size, brightness, chroma and hue. Second, a communication system needs to be examined for signals that are expressed in different modalities, for example visual, acoustic, tactile or chemical. Finally, the signalling environment of a species has a high impact on signal transmission and detection efficacy (Bro-Jørgensen, 2010). Acoustic signals might be adequate for long-range communication or communication at night, while most visual signals require sufficient light and absence of obstacles between sender and receiver, as shown experimentally in the display behaviour of male wolf spiders, *Schizocosa floridana* (Rosenthal, Wilkins, Shizuka, & Hebets, 2018). Changes in signalling environments can cause the sender to switch communication channel/modality and thus facilitate multimodal signalling.

Most seabirds are long lived and both males and females signal to attract mates. Their breeding behaviour is energetically costly; one of the longest breeding cycles of all bird species is that of king penguins, extending over more than 13 months (Barrat et al., 1976; Descamps, Gauthier-Clerc, Gendner, & Maho, 2002). Until the chick is fully able to regulate its own body temperature (ca. 30 days), both parents alternate in caring for the egg or the chick in the colony and undergo prolonged fasting periods (Descamps et al., 2002; Stonehouse, 1960). Adults may abandon the egg or the chick to forage at sea before their partner returns, leading to breeding failure (Gauthier-Clerc, Le Maho, Gendner, Durant, & Handrich, 2001; Groscolas et al., 2000; Olsson, 1997). The severe depletion of energy reserves during fasting and the experience needed to synchronize alternating parental care lead to a late age of successful first reproduction in king penguins (average age of 5 years). Younger and inexperienced birds are rarely successful (Le Bohec, 2007). Le Vaillant et al. (2012) showed that age is related to foraging efficiency, with older birds gaining more body mass than younger individuals.

When king penguins have completed their moult, replenished their energy reserves at sea and returned to their breeding ground, both sexes engage in courtship behaviour (see Jouventin, 1982). Display calls are produced by single males and females outside the

breeding colony. If a male and a female approach each other, the 'face-to-face' behaviour can be observed during which both individuals stretch upwards, move their heads from side to side and often produce calls as well. Two birds are sometimes joined by one or more other individuals; trios are formed most often consisting of two males competing for one female (Keddar, Andris, Bonadonna, & Dobson, 2013). However, groups of more than three birds and competitive interactions between females do occur (H.J. Kriesell, personal observation). After this ritualized courtship behaviour involving the display of ornaments and vocalizations, and interactions with numerous individuals, pairs settle in the colony.

Kraaijeveld (2003) argued that sexually monomorphic ornamentation as signals of individual quality might evolve in a species with high divorce rates, where individuals must find a new mate in a short period of time to increase their chances of breeding success. In a review, Dubois, Cézilly, and Pagel (1998) ranked king penguins among the eight seabird species with the highest divorce rates, estimated to be between 75% (Weimerskirch, Stahl, & Jouventin, 1992) and 63% (Toscani, 2010). Considering the pressure on king penguins to find new mates and the high cost for both sexes of raising a chick, we might expect that king penguins would produce communication signals whose parameters are correlated with an individual's age, and thus potentially signal an individual's ability to successfully raise a chick. The only study focusing on age signalling in king penguins showed that beak and ear patch ornamentation parameters are correlated with age in birds between 2 and 6 years of age (Nicolaus et al., 2007). However, this is the age during which king penguins are only just about to start breeding (Weimerskirch, et al., 1992; Le Bohec, 2007).

King penguins of both sexes have identical sexual ornaments: (1) bright yellow-orange beak spots on both sides of the mandibles made of keratin layers that also reflect in the ultraviolet (UV) range (Dresp, Couchoux & Jouventin, 2005; Jouventin, Nolan, Örnborg, & Dobson, 2005), (2) yellow-orange ear patches on both sides of the head and (3) a breast patch of a brown to light yellow colour gradient resulting from pterin-like pigments (Hill & McGraw, 2006a; McGraw et al., 2004). The beak spot and the ear patch are important signals during mate choice: both sexes with experimentally reduced ear patches or reduced UV-reflecting beak spots took significantly longer to find a mate (Jouventin, Nolan, Dobson, & Nicolaus, 2008; Nolan et al., 2010; Pincemy, Dobson, & Jouventin, 2009). Viblanc et al. (2016) showed that colourful ornaments act as honest signals of individual quality in king penguins.

Our knowledge of king penguin vocalizations mainly concerns their function in individual recognition. Previous acoustic studies presented experimental evidence that king penguins produce individually distinct display calls that are used for individual identification and kin recognition (Aubin, Jouventin, & Hildebrand, 2000; Jouventin, Aubin & Lengagne, 1999). Calls are frequently produced by both sexes during courtship (Jouventin, 1982) and are sex specific (Kriesell et al., 2018), but we do not currently understand the specific role of king penguin vocalizations during mate attraction. Age-specific vocal features in king penguins, and thus the age-related information that calls may convey, have remained unstudied. Interestingly, courtship areas in king penguin colonies are visited by a wide age range of individuals. We can identify three age classes that vocalize (1) 1-year-old fledglings (subsequently referred to as chicks) that are thermally independent and about to leave for the sea for the first time, (2) 2-year-old juveniles returning from their first long foraging trip at sea and (3) sexually mature adults of differing age and breeding experience searching for partners for the upcoming breeding season. While only adults engage in courtship behaviour that leads to breeding (Jouventin, 1982; Keddar, Jouventin & Dobson, 2015), fledglings and juveniles

can be observed to sporadically engage in parading behaviour as well.

In this study, we investigated whether ornamentation and/or call parameters are related to age class (i.e. chicks, juveniles, adults) and age in adult birds. King penguin calls consist of syllables that are separated by strong fluctuations in frequency and amplitude (Lengagne, Lauga, & Aubin, 2001). We previously identified two syllable types ('A' and 'B'; Kriesell et al., 2018), and showed that adult male and female king penguins can be distinguished with 100% accuracy by the syntax of their syllable patterns, with females producing a 'BAB' pattern and males 'AAB'. Here, we used the same approach to investigate whether the same syllable types and a sex-specific syntax exist for chicks and juveniles.

Given the high divorce rate, the high investment in reproduction of both sexes, the low success of young breeders and the display areas being occupied by all age classes, we hypothesized that both ornamentation and call components are correlated with age class in king penguins. Age-assortative pairing has, for example, been observed in flamingos, *Phoenicopterus ruber roseus* (Cézilly, Vincent, Tourenq, & Johnson, 1997), and observations from long-term monitoring suggest that king penguins pair with individuals of similar age (Le Bohec & Kriesell, n.d.). We hypothesized that ornaments and call parameters are signals of age in adult birds.

## METHODS

### Study Area

The study was conducted at two field sites and during the field seasons of November 2015 to April 2016 and November 2016 to January 2017. The first field site was La Grande Manchotière king penguin colony (ca. 16 000 breeding pairs, Delord, Barbraud, & Weimerskirch, 2004) in La Baie du Marin (BDM) at Possession Island, Crozet Archipelago (46°25'S, 51°45'E). In the scope of a long-term monitoring project that started in 1998 (Programme IPEV 137-ANTAVIA), king penguins are implanted with radio frequency identification (RFID) tags in their first year of life to allow monitoring of known-age birds. The studied subcolony is only accessible for the birds by three pathways, each of which is equipped with two antennas that are buried at a depth of about 20 cm in the ground. This permanent automated system allows the identification of microtagged individuals as they cross the antennas to enter or leave the colony (see Gendner, Gauthier-Clerc, Le Bohec, Descamps, & Le Maho, 2005). King penguins at BDM have been exposed to human presence since the early 1960s. Birds likely either habituated to human stressors or tolerant phenotypes have been selected for in this colony (Viblanç, Smith, Gineste, & Groscolas, 2012). The second

study site was Cap Ratmanoff (CR) colony (49°12'N, 70°33'E) in the Kerguelen Archipelago, consisting of approximately 100 000 king penguin breeding pairs (Keddar et al., 2015). Long-term monitoring of this colony was established in 2011, where microtagged king penguins are identified by automated systems temporarily deployed at the access pathways of the birds to their natal and breeding zones.

### Study Birds

We collected data from all three age classes: (1) fledglings (1 year old) that had just moulted their brown down to waterproof feathers, (2) juveniles (2 years old) that had just returned from a year-round foraging trip at sea and still had old plumage, i.e. had not yet moulted white or very light yellow ear patches (Jouventin & Dobson, 2018), and (3) adults (3 years and older) with renewed adult plumage of the year. The adult group contained birds of known age that were breeders in the ANTAVIA subcolony and birds of unknown age parading at the beach of BDM. At the beginning of each breeding event, adult king penguins undergo a drastic moult on land during which all plumage, including the ornaments, is renewed (Groscolas & Cherel, 2007). The moult is followed by the parading phase. Juveniles arrive later in the season at the colony (Saraux, Viblanç, Hanuise, Le Maho, & Bohec, 2011), which meant that few juveniles were present at the colony during our study period and that they still had old, not-yet-moulted plumage. Therefore, their ornamentation parameters could not be compared with those of moulted birds and only those of the other two age classes, chicks and adults, were compared. This restriction did not apply to acoustic parameters and all three age classes were included in the acoustic analyses. Acoustic recordings of males and females were all recorded at BDM for adults, and at BDM and CR for juveniles and chicks. The sample sizes are summarized in Table 1.

### Ornamentation Measures and Data Processing

Birds were captured outside the breeding areas to avoid disturbance of breeding individuals. During each capture event, a hood was placed immediately over the head of the bird covering its eyes to ensure that the bird stayed as calm as possible. Although sex identification took place in the field by listening to the individual's call, a 3 ml blood sample was taken from the brachial vein to allow for confirmation by molecular sexing. For each bird, we measured the size of the flippers and the beak using a metal ruler (Appendix Fig. A1a, b). We calculated a structural size index (SSI) by performing a principal component analysis on flipper and beak length, two correlated measurements that indicate the size of a penguin

**Table 1**  
Sample size, i.e. number of birds from which visual and/or acoustic data were obtained

Maturity class	Sex	Colony	Moulted	Age (years)	Breeding status	Ornaments	Calls
Chick	M	BDM	Yes	1	NB	28	11
Chick	F	BDM	Yes	1	NB	21	9
Chick	M	CR	Yes	1	NB	0	7
Chick	F	CR	Yes	1	NB	0	7
Juvenile	M	BDM	No	2	NB	0	8
Juvenile	F	BDM	No	2	NB	0	10
Juvenile	F	CR	No	2	NB	0	2
Adult (unknown age)	M	BDM	Yes	≥ 3	PAR	47	41
Adult (unknown age)	F	BDM	Yes	≥ 3	PAR	26	31
Adult (known age)	M	BDM	Yes	4–18	B	69	17
Adult (known age)	F	BDM	Yes	4–18	B	49	10

Data are given for each age class (chicks, juveniles, adults), sex (F = female, M = male), king penguin colony (BDM = Baie du Marin colony in Crozet, CR = Cap Ratmanoff colony in Kerguelen), moulted status (Yes = freshly moulted bird, No = old plumage) and breeding status (NB = nonbreeder, PAR = parading, B = breeder).

reliably, can easily be taken easily in the field and show a high repeatability (Fahlman et al., 2006; Saraux et al., 2011). The SSI was equivalent to the first principal component (first eigenvector PC1), which explained 76% of the variation in beak size and flipper length. Using a tape measure, we measured two ornamental features to the nearest 1 mm: (1) the apical distance of head ornaments as the smallest distance between the yellow patches on top of the head ('apical'; Appendix Fig. A1c), and (2) the yellow-feathered ear patch size (Dobson, Couchoux, & Jouventin, 2011; Viblanc et al., 2016). The shape of the latter was considered an ellipse and we measured the vertical and, in a perpendicular line, the horizontal length of the ear patches (Appendix Fig. A1d). We subsequently calculated the ear patch size:

$$\text{Surface (ear patch)} = \frac{\pi L W}{4}$$

where  $L$  = length and  $W$  = width of the ear patch.

Using a USB2000 spectrophotometer and a PX2 pulsed-xenon light with a 200  $\mu\text{m}$  fibre-optic probe that was calibrated against a white standard (WS-1SL, Ocean Optics, Dunedin, FL, U.S.A., Spectralon), the reflectance of the ear patches was measured at a 90° angle using an adapter and the reflectance of the beak spots at a 30° angle window without adapter.

While the beak spot is a hard surface, the ear patch feathers cave in under pressure of the optic fibre and reflectance spectra differed according to different pressure applied resulting in nonrepeatable measurements: repeatability for beak spot measurements without an adapter ranged from 0.66 to 0.87, for ear patch measurements without an adapter from 0.25 to 0.46 and with an adapter from 0.53 to 0.67. We therefore used an adapter that consisted of a tube into which the optic fibre was inserted, for all ear patch measurements. We did not take measurements of the breast patch, because it would have prolonged the handling time and colour measurements were not repeatable within and between individuals: the breast colour gradient was found to change depending on the extent to

which the bird would stretch, and no fixed measurement points could be established.

Measurements of each ornament (left and right side) were repeated three times and the spectra averaged. The repeatability of the ear patch colour measurements was calculated using the RPackage rptR (Nakagawa & Schielzeth, 2010). Interindividual variability was higher than intraindividual variability between the six repeated measurements described above. Each bird was released after approximately 20 min and marked with a number using human hair dye (Franck Provost, blue-black 2.1, Paris, France) allowing each bird to be identified from a distance without requiring capture.

Processing of ornamentation data involved four steps: (1) averaging the six measurements of each ornament, (2) spline smoothing the spectra, (3) rescaling the spectra and (4) calculating all colour parameters for which we used functions given by Montgomerie (2006).

The colour of the king penguin beak relies on two different mechanisms: a structural colour reflects UV (between 280 to 490 nm with a peak at around 370 nm) and a pigment-based yellow-orange (YO) part results from carotenoid absorption (from 490 to 700 nm; Dresp, Jouventin, & Langley, 2005; McGraw et al., 2007). The pigments of the ear patch absorb UV-light but reflect YO (measured over 280 to 700 nm). For these three spectral regions (UV beak, YO beak and YO ear patch), we calculated the brightness, hue and chroma (Hill & McGraw, 2006a). We chose to calculate the parameters of the signal over a total spectral range of 280 nm–700 nm. All measured colour parameters are summarized in Table 2.

#### Acoustic Recordings and Data Processing

Chicks, juveniles and parading adults of unknown age were recorded outside the breeding areas of BDM and CR colonies. Data were collected in favourable weather conditions (wind speed < 4 m/s and no rain) during daylight hours (0500–1900 hours). In order not to disturb breeding adults of known age at BDM, acoustic recordings

**Table 2**  
Ornamentation parameters, their abbreviations, units, type of ornament measured and at which wavelengths, calculated for chick and adult king penguins from BDM, Crozet

Parameter	Abbreviation	Unit	Ornament	Measured at/between wavelength/s (nm)	Description
Apical distance	Apical	Mm	Ear patch	NA	Measured distance of head ornaments as the smallest distance between the yellow patches on top of the head
Border	B <sub>border</sub>	Nm	Beak	400–600	Wavelength at minimum reflectance between UV and YO part of the beak spectrum
Brightness beak	Bright <sub>beak</sub>	Area	Beak	280–700	Sum reflectance
Brightness ear patch	Bright <sub>EP</sub>	Area	Ear patch	280–700	Sum reflectance
Brightness UV	Bright <sub>UV</sub>	Area	Beak	280–B <sub>border</sub>	Sum reflectance
Brightness YO	Bright <sub>YO</sub>	Area	Beak	B <sub>border</sub> –700	Sum reflectance
Chroma ear patch	Chroma <sub>EP</sub>	unitless	Ear patch	280–700	(Maximum reflectance of ear patch–minimum reflectance)/mean YO
Chroma UV	Chroma <sub>UV</sub>	unitless	Beak	280–B <sub>border</sub>	(UV maximum – UV minimum)/mean Brightness UV
Chroma YO	Chroma <sub>YO</sub>	unitless	Beak	B <sub>border</sub> –700	(YO maximum – YO minimum)/mean Brightness YO
Ear patch surface	Ear patch size	Mm	Ear patch	NA	The longer and in a perpendicular line the larger of the ear patches
Hue ear patch	Hue <sub>EP</sub>	Nm	Ear patch	280–700	Wavelength for which the reflectance is halfway between its maximum and minimum
Hue UV	Hue <sub>UV</sub>	nm	Beak	280–B <sub>border</sub>	Wavelength at maximum reflectance
Hue YO	Hue <sub>YO</sub>	nm	Beak	B <sub>border</sub> –700	Wavelength for which the reflectance is halfway between its maximum and minimum intensity
Inflection point chicks' beak spot	Max <sub>B</sub>	unitless	Chick's beak	NA	Wavelength at which the derivative is the highest Max <sub>B</sub>
Inflection point ear patch	Max <sub>EP</sub>	unitless	Ear patch	NA	Wavelength at which the derivative is the highest Max <sub>EW</sub>
UV maximum	UV <sub>max</sub>	% reflectance	Beak	280–B <sub>border</sub>	Maximum reflectance of UV part of the beak spectrum
YO Maximum	YO <sub>max</sub>	% reflectance	Beak and Ear patch	680–700	Mean reflectance of the YO part of the beak or ear patch spectrum

YO = yellow-orange coloration.

were only collected from individuals breeding on the periphery of the colony, during reunions of partners or parents with chicks. King penguin vocalizations were recorded using an omnidirectional microphone (Sennheiser, K6-me62, frequency response: 20–20 000 Hz  $\pm$  2.5 dB) mounted on the end of a 2 m rod and connected to a Marantz PMD 661 digital recorder (frequency response: 20 Hz–24 kHz  $\pm$  1 dB; sampling frequency: 44.1 kHz). Calls were recorded with the microphone approximately 1 m from one side of the bird's beak. A combined foam and fur windshield was used to reduce wind noise. The rod allowed for approximately 3 m between the recording person and the focal bird. This set-up reduced disturbance, since the focal birds were mobile, and we followed them to ensure approximately the same distance between the penguin and the microphone. The average recording time was 26.1 min for adult birds, 17.9 min for juveniles and 14 min for chicks. A minimum of three calls were recorded per individual with an average of eight calls for adults, four calls for juveniles and five calls for chicks.

Recordings were analysed using Avisoft SASLab Pro, version 5.2.09 (Avisoft Bioacoustics, Berlin, Germany). They were downsampled to 22.05 kHz prior to the analysis and high-pass filtered at 0.20 kHz to remove low-frequency background noise (see Supplementary material for calls of an adult male and a female king penguin).

Apart from identifying the syllable types and a potentially sex-specific syntax in all study birds, we acquired duration measurements using the waveform of the entire call and of the first and an intermediate A-type syllable. The energy quartiles, frequency of maximum amplitude ( $F_{\max}$ ) and the fundamental frequency F0 (the lowest frequency) and its modulation were measured on the spectrogram (FFT length = 1024, 98.43% overlap, Blackman window) using the free reticule cursor (see Table 3 for a summary of acoustic parameters).

### Statistical Analyses

All statistics were computed using R software, version 3.3.1 (R Core Team, 2013). The sample size of birds for which we had both colour and call measurements ( $N_{\text{males}} = 16$ ,  $N_{\text{females}} = 11$ ) was not of sufficient statistical value to analyse them jointly. We therefore analysed visual and acoustic features separately. As a first step, we standardized, i.e. subtracted the mean from each value and divided by the standard deviation, for all variables to be able to compare the estimates within models.

### Sex difference and breeding status

Independently for ornamentation and acoustics, we investigated differences between (1) females and males and (2) breeders and parading birds using a Welch's *t* test. We applied a false discovery rate correction (FDR; Benjamini & Hochberg, 1995), which reduced the number of false negatives.

### Differences between age classes

We used a Welch's *t* test with FDR correction to test for differences in ornamentation parameters between king penguin

chicks and parading adults that had all completed their moult. We compared ornamentation data of chicks and parading adults since (1) we were interested in how the birds distinguish maturity classes in the context of mate choice and breeding birds had already found a partner, (2) parading adults and chicks occur in the same parading areas, while breeding adults are in the colony and only leave to go to sea, and (3) chicks and parading adults have both just completed the moult and are thus comparable in terms of their stage of moult. We pooled data from BDM and CR for juveniles as well as chicks (Welch's *t* test with FDR correction, all  $P > 0.6$ ) since we did not find differences for any of the acoustic parameters between the colonies for both age classes. To account for potential body size effects when comparing ornament sizes (in king penguins; Viblanc et al., 2016) and fundamental frequency (in banded penguins, *Spheniscus*; Favaro, Gamba, Gili, & Pessani, 2017) between age classes, we used linear models considering SSI as a covariable. Because all fundamental frequency (F0) measurements were highly correlated, we used the mean of the F0 measurements (start, inflection point, end) of the first and middle syllables to decrease the number of tests and limit type I errors due to multiple testing. We ensured that residuals followed a normal distribution using qqplots (opposing theoretical quantiles to sample quantiles).

To test whether ornamentation or acoustic parameters encode information about age class and age, we used a cross-validation procedure and ran generalized linear models (GLMs) separately for each sex, where age was specified as the fixed factor and models were run with either ornamentation or acoustic parameters as dependent variables. Each model was run 1000 times. The data set was split into a training and a test subset for each run. Training subsets were taken as stratified random samples from the empirical probability distribution using the same proportion of males and females, and the remaining individuals were used to test the predictive power of the models in the test sample. Because our data did not show a specific distribution pattern, we ran the models with different link functions and used forward and backward stepwise selections based on the AIC criterion to find the best-fit model. However, all models failed to fit the data, and we therefore used classification trees to investigate nonlinear relationships with age class (De'Ath & Fabricius, 2000) and the optimal response index (ORI, Planas-Bielsa, Ortega, Eckbo, & Le Bohec, n.d.) to investigate nonlinear relationships with continuous age.

We used classification trees to predict age classes with our set of ornamentation and acoustic parameters (Appendix Fig. A2). While the relation between the variables can be nonlinear and complex, the classification trees can accommodate a large number of variables and do not suffer from high covariances between variables (De'Ath & Fabricius, 2000; Hayes, Usami, Jacobucci, & Mcardle, 2015). While one aim is to achieve a low misclassification rate for a given data set, overfitting must be avoided to ensure model robustness (see review of tree model selection criteria by Song &

**Table 3**

Summary of acoustic parameters measured from chick, juvenile and adult king penguin calls

Call parameter domain	Call parameters measured
Syllables	Identification of groups of syllables 'BAB' and 'AAB'
Time (s)	$T_{\text{tot}}$ : total call duration $T_1$ : syllable duration of first syllable $T_3$ : syllable duration of intermediate syllable Tempo: mean duration of the first two type 'A' syllables within a call
Frequency (kHz)	F0: fundamental frequency measured at the start, inflection point and end of the first and intermediate syllable $F_{\max}$ : frequency of maximum amplitude of first and intermediate syllable
Energy (kHz)	Energy quartiles $Q_{25}$ , $Q_{50}$ , $Q_{75}$ : frequency values that delineate the quartiles in energy measured at first syllable and intermediate syllable $E_{3000}$ : energy below 3000 Hz measured at first and intermediate syllable

Lu, 2015). We therefore applied stopping rules: (1) a minimum number of observations required before splitting or including the next factor is allowed (minimum node observations), (2) a fixed depth that controls the maximum size of a tree (the minimum is zero in a tree, which consists of only one variable, and the maximum is 30), (3) smallest cross-validation error based on a 10-fold cross validation, (4) postpruning on the initial tree choosing the complexity parameter (CP) associated with the smallest cross-validation error. The CP is essential for controlling tree size since it defines whether a split into a new node is attempted or not, depending on whether it contributes to a better model fit. This allowed us to identify the most parsimonious tree, i.e. one with a minimal number of variables while achieving the lowest misclassification rate of the target variable. We subsequently applied the classification tree on randomly generated data and compared the misclassification rate obtained with our real data.

The ORI approach can be understood as an alternative to GLMs and uses rank correlations to detect nonlinear relationships between explanatory and dependent variables when the link function (or the error distribution) is unknown. The output of the ORI analysis is a vector with the relative importance of the explanatory variables to explain the response. We performed ORI on four different models: age as a function of ornament parameters (separately for each sex) and as a function of acoustic parameters (separately for each sex). For each model, we calculated the optimal rank correlation and estimated a numerical *P* value by generating a large number of random uncorrelated observations of the same size and estimating the distribution of maximum rank correlation for the null model. Since we had only a few data points, we had to reduce the dimensionality of the model and only chose three variables per model to avoid overfitting. We did this by removing highly correlated variables (Spearman correlation > 0.9).

#### Ethical Note

This study was approved by the French ethics committee (APAFIS#4897–2015110911016428) and the French Polar Environmental Committee (TAAF permit #2015-105 & 2016-76) and conducted in accordance with its guidelines.

Several measures were taken to minimize disturbance and stress of the study birds. Acoustic measurements were carried out using a 2 m pole to allow for at least 3 m between the recording person and the animal. During each capture event, a hood was placed immediately over the head of the bird covering its eyes to ensure that the bird stayed as calm as possible, and the manipulation was carried out silently. Handling was conducted in a shelter close to the colony to limit the time and stress associated with transport, and to be protected from the weather. The handlers were constantly watching for external behavioural signs of stress and overheating of the bird (for example open beak) and, if necessary, birds would have been released immediately. Handling time for measurements of the birds was minimized and kept to < 30 min per bird. After handling, the bird was placed back at the location of capture to allow it to orient itself as quickly as possible. The bird was observed from a distance for several minutes

after release to ensure that it resumed normal activity. No injuries or deaths occurred during or after handling the birds.

## RESULTS

### Ornamentation Parameters

#### Differences in sex and breeding status

Within the group of breeding adults, females did not differ significantly from males in any of the colour parameters (Welch's *t* test: all *P* > 0.05).

In the parading adult group, females had a brighter YO beak spot (Bright<sub>YO</sub> mean ± SD: 13 596 ± 921; Welch's *t* test: *t* = 2.84, *P* = 0.007) and consequently a higher overall brightness of the beak (Bright<sub>beak</sub> 18 774 ± 1600; Welch's *t* test: *t* = 3.40, *P* = 0.02) than males (Bright<sub>YO</sub> 12 605 ± 1557; Bright<sub>beak</sub> 17 509 ± 2120), while parading males showed a higher Chroma<sub>UV</sub> (1.97 ± 0.18; Welch's *t* test: *t* = 4.06, *P* = 0.002) than females (1.82 ± 0.13). Parading females had brighter beak spots (Bright<sub>beak</sub>, Bright<sub>UV</sub> 5180 ± 1058, Bright<sub>YO</sub>), a higher saturated UV part of the beak (Chroma<sub>UV</sub> 2.03 ± 0.15), and the border between the UV and YO part of the beak was shifted towards the YO spectrum, i.e. the B<sub>border</sub> value (483 ± 3 nm) was higher than in breeding females (Bright<sub>beak</sub> 16 027 ± 1449, Bright<sub>UV</sub> 4153 ± 727, Bright<sub>YO</sub> 11 879 ± 1108, Chroma<sub>UV</sub> 1.82 ± 0.13, B<sub>border</sub> 481 ± 5 nm; Welch's *t* test: all *P* < 0.0001). The differences between parading and breeding males concerned similar parameters, but the differences were less evident than for females. Parading males showed brighter beak spots (Bright<sub>beak</sub> 17 486 ± 2135; Welch's *t* test: *t* = 4.07, *P* = 0.03; Bright<sub>UV</sub> 4880 ± 1027; Welch's *t* test: *t* = 3.78, *P* = 0.001; Bright<sub>YO</sub> 12 609 ± 1573; Welch's *t* test: *t* = 3.10, *P* = 0.002), a higher saturated UV part of the beak (Chroma<sub>UV</sub> 2.04 ± 0.12; Welch's *t* test: *t* = -2.63, *P* = 0.01), and a higher UV maximum of the beak spot (UV<sub>max</sub> 16.88 ± 3.23; Welch's *t* test: *t* = 2.58, *P* = 0.03) compared to breeding males (Bright<sub>beak</sub> 16 047 ± 1573, Bright<sub>UV</sub> 4248 ± 708, Bright<sub>YO</sub> 11 802 ± 1153, Chroma<sub>UV</sub> 1.97 ± 0.18, UV<sub>max</sub> 15.47 ± 2.57).

#### Differences between age classes

King penguin chicks and parading adults differed significantly in all colour parameters (Welch's *t* test with FDR correction: all *P* < 0.0001). The beak spots of chicks are black and did not reflect in the UV spectrum (UV<sub>max</sub> was on average 4.77% (± 2.84) for chicks and 16.80% (± 3.12) for parading adults). The ear patches of chicks were light yellow compared to the yellow-orange ear patches of adults, and this difference was quantified in the difference of the Hue<sub>EP</sub> measurements (chicks: 495 ± 7 nm; parading adults: 527 ± 14 nm). Adults also had larger ear patches and smaller apical distance than chicks in the linear models, taking the individuals' SSI as a covariable into account (Table 4).

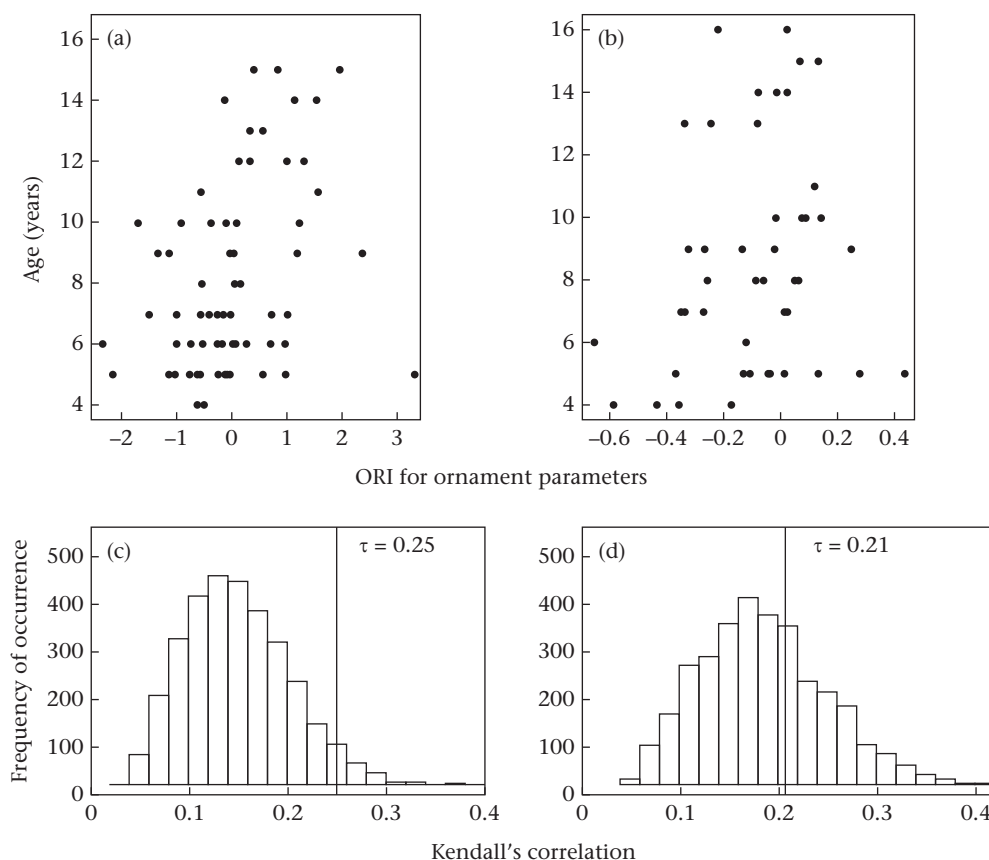
Using any of the beak spot parameters, the classification tree consisted of only one node, since the two age classes could be

**Table 4**

Model parameter estimates for the linear models explaining the variation in ear patch size and apical distance by age class and structural size index (SSI)

Model	Variable	Model effect estimate + SE	<i>T</i>	<i>P</i>
Ear patch size ~ Age-class + SSI	Intercept	1426.76 + 33.58	42.49	< 0.0001
	Age-class (adults vs chicks)	158.39 + 46.58	3.40	< 0.001
	SSI	79.29 + 18.95	4.18	< 0.0001
Apical ~ Age-class + SSI	Intercept	39.90 + 0.77	51.53	< 0.0001
	Age-class (adults vs chicks)	-3.45 + 1.07	-3.21	0.002
	SSI	0.15 + 0.44	0.34	0.732

*N*<sub>chicks</sub> = 49; parading *N*<sub>adults</sub> = 73 king penguins.



**Figure 1.** (a, b) Optimal response index (ORI) for king penguin ornament parameters for which Kendall's correlation between the index variables and the response variable age was maximized. The indexes were calculated separately for (a) males ( $\tau = 0.25$ ,  $N = 69$ ) with the variables  $Bright_{UV}$ , Apical distance and  $Chroma_{UV}$  and (b) females ( $\tau = 0.21$ ,  $N = 49$ ) with the variables  $UV_{Max}$ ,  $Bright_{UV}$  and  $Chroma_{UV}$ . (c, d) Histogram with the distribution of Kendall's tau with random ornamentation data with 3000 Monte Carlo simulations for (c) males and (d) females. The vertical lines mark the Kendall's tau that we achieved with our data for each sex.

separated by only one of these beak colour variables alone, resulting in 100% correct classifications ( $N_{chicks} = 49$  and  $N_{adults} = 118$ ). The inflection point ear patch ( $Max_{EP}$ ) was the variable that resulted in only one chick being misclassified as an adult and thus in a correct overall classification of 99.4%.

#### Age of adults and ornament parameters

All colour parameters in the GLM explaining age of adults were nonsignificant for both females and males. We obtained low correlations between predicted and real age for adult king penguins in the ORI estimate:  $\tau = 0.25$  for males, aged between 4 and 15 years (Fig. 1a), and  $\tau = 0.21$  for females, aged between 4 and 16 years (Fig. 1b). The correlation between age and ornamentation variables using random data was significantly different from the correlation calculated with the real data for males ( $P = 0.03$ ; Fig. 1c), but not for females ( $P = 0.36$ ; Fig. 1d).

For males,  $Bright_{UV}$  had the highest impact on the ranking, while  $UV_{Max}$  was most important in the female model (Table 5). Notably, only one ear patch parameter (Apical) improved the model fit, whereas the rest of the variables were measures of the UV part of the beak spot.

#### Acoustic Parameters

#### Differences in sex and breeding status

Chicks, juveniles and parading adults showed sex-specific syllable patterns: 'AAB' for males and 'BAB' for females, allowing for a clear separation between sexes (see Fig. 2). We thus kept males and

females separated for the subsequent analyses of acoustic parameters.

Parading birds produced on average only  $5.4 \pm 1.6$  syllables in a call, while breeding birds produced  $8.1 \pm 1.7$  syllables. Since call duration is positively related to the number of syllables in a call, it differed significantly between calls of breeding adults and parading adults (Welch's  $t$  test:  $t = 0.69$ , FDR-corrected  $P = 0.0002$ ), with parading birds' calls being shorter ( $2.21 \pm 0.60$  s) than calls of breeding birds ( $3.91 \pm 0.67$  s).

#### Differences between age classes

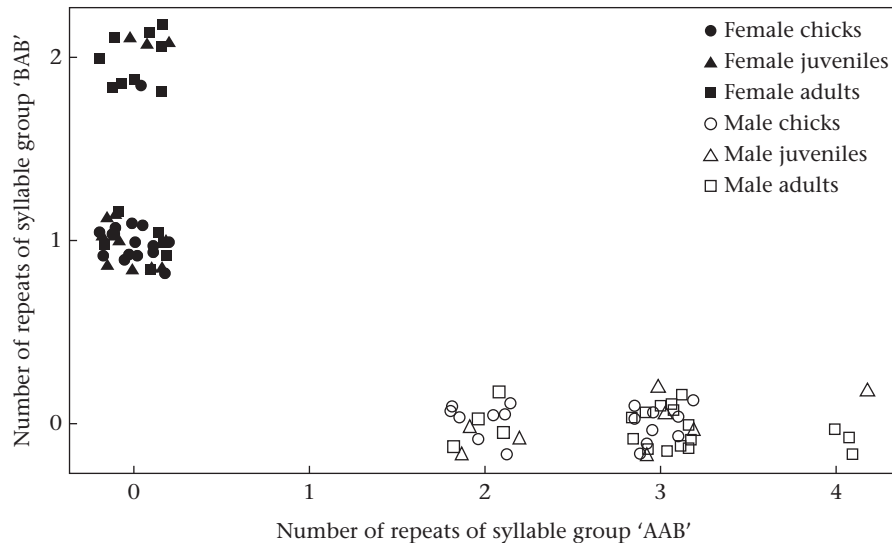
When we applied classification trees separately for each sex using the selection criteria summarized in Table 6, the best classification trees, i.e. with the highest classification rate while using the lowest number of variables, used only four acoustic variables per sex to identify age class (Table 6, Appendix Fig. A2).

**Table 5**

$\beta$  coefficients of the ornament features used in the ORI analysis that maximize Kendall's correlation between the linear index of these variables and adult age (one model per sex)

Sex	Variable	B
Male	$Bright_{UV}$	-0.955
	Apical	0.263
	$Chroma_{UV}$	0.140
Female	$UV_{Max}$	0.782
	$Bright_{UV}$	-0.559
	$Chroma_{UV}$	-0.276





**Figure 2.** Scatterplot for call syllable patterns of chicks ( $N = 33$ ), juveniles ( $N = 21$ ) and parading adults ( $N = 35$ ). Calls that overlapped each other or for which the last part was masked or interrupted were excluded from this syllable pattern analysis leading to smaller sample sizes. A 'jitter' function was applied to make the individual data points visible.

The male and female trees shared two of the four selected variables, and all variables were frequency and energy quartile parameters (Table 6, Appendix Fig. A2). The main classifier between chicks versus juveniles and adults was the fundamental frequency ( $F_0$  inflection first syllable for females and  $F_{00}$  inflection intermediate syllable for males) being higher for chicks (higher than 657 Hz for female chicks and 570 Hz for male chicks).

Given that 58 of the 84 males in the study were in the adult age class, a correct classification of 31% can be achieved by randomly classifying all individuals into the adult category. For example, classifying all female birds, i.e. chicks, juveniles and adults, into the adult category gives a correct classification rate of 41%. The aim therefore is to outperform these random classifications with the classification tree using the real variables. Four chicks, three juveniles and one adult out of 69 females were misclassified (correct classification rate of 88%), while two juveniles and three adults out of 84 males were misclassified (correct classification rate of 94%). To achieve the same correct classification rate of 94% using random data, the algorithm included 14 variables (CP = 0.002, depth = 10, minimum node observations = 4), and thus performed worse than the real data model. Similarly, for females, the random data tree was composed of 12 variables (CP = 0.002, depth = 5, minimum node observations = 2).

Fundamental frequency parameters in both trees were the best predictor, i.e. the root node. Accordingly, mean fundamental frequency differed significantly between age classes and sexes when structural size was taken into account as a covariable (linear model:  $F_{77} = 28.96$ ,  $P < 0.0001$ ), with chicks producing calls of

higher mean fundamental frequency than juveniles, and juveniles than adults. Although females produced higher mean fundamental frequencies than males, the structural size of the bird had no effect (Table 7).

#### Age of adults and acoustic parameters

Applying the ORI, we obtained a Kendall's correlation of  $\tau = 0.62$  between predicted and real age for males (5–14 years; Fig. 3a) and  $\tau = 0.93$  for females (4–17 years; Fig. 3b), using only three variables (Table 8). The probability of achieving the same Kendall's correlation was  $P = 0.022$  for males (Fig. 3c) and  $P = 0.001$  for females (Fig. 3d), and thus confirmed that the correlation we obtained with our data was related to an existing nonlinear relation between the acoustic predictor variables and age.

## DISCUSSION

In this study, we showed that age class in king penguins was encoded bimodally, that is, in vocalizations and in ornament parameters, and that adult age was related to acoustic parameters, but not to ornament parameters. We found that syllable syntax encoded sex for all age classes.

#### Ornaments and Calls Signal Age Class

Chicks had no YO or UV reflection allowing a 100% classification rate between chicks and adults. Adults had bigger ear patches than chicks (corrected by the size of the bird). Similar results were found

**Table 6**  
Model selection criteria applied in the classification tree

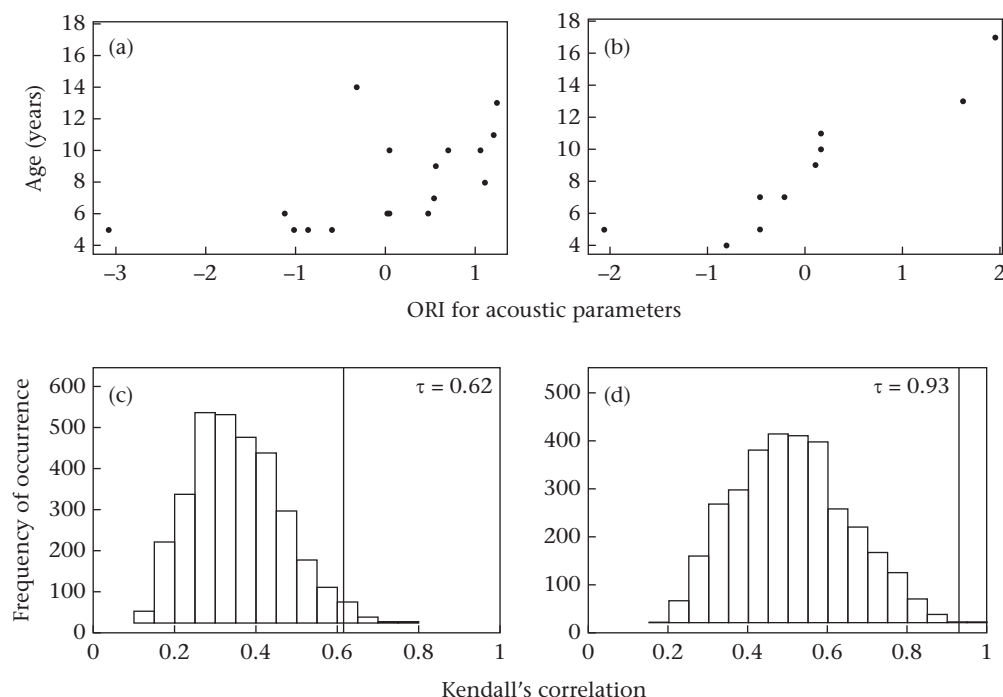
Target variable	Independent variables	Sample sizes	Minimum node observations	Tree depth	Pruned CP	Accuracy (in %)
Male age class	$F_0$ inflection intermediate syllable	Chicks = 18	7	9	0.05	96
	$Q_{25}$ first syllable	Juveniles = 8				
	$Q_{50}$ first syllable	Adults = 58				
Female age class	$Q_{25}$ intermediate syllable	Chicks = 16 Juveniles = 12 Adults = 41	8	5	0.06	88
	$F_0$ inflection first syllable					
	$Q_{25}$ first syllable					
	$Q_{50}$ first syllable					
	$F_0$ inflection intermediate syllable					

CP = complexity parameter of pruned tree. Accuracy is calculated as the number of correct classified individuals divided by the total sample size for each sex.

**Table 7**  
Model parameter estimates for the linear models explaining the variation in fundamental frequency by age-class, sex and structural size index (SSI)

Model	Variable	Model effect estimate + SE	T	P
Mean fundamental frequency ~ Age class + Sex + SSI	Intercept	534.67 + 11.69	45.74	< 0.0001
	Age class (chicks vs juveniles)	57.42 + 14.73	3.90	< 0.001
	Age class (adults vs juveniles)	-44.86 + 13.33	-3.36	0.001
	Sex (males vs females)	-29.85 + 10.22	-2.92	0.005
	SSI	-6.54 + 6.28	-1.04	0.300

$N_{\text{chicks}} = 30$ ,  $N_{\text{juveniles}} = 18$ ,  $N_{\text{adults}} = 34$  king penguins.



**Figure 3.** (a, b) Optimal response index (ORI) for acoustic parameters for which Kendall's correlation between the index variables and the response variable age was maximized. The indexes were calculated separately for (a) males ( $\tau = 0.62$ ,  $N = 17$ ) with the variables Tempo, Energy < 3000 Hz, Max frequency first syllable and (b) females ( $\tau = 0.93$ ,  $N = 10$ ) with the variables Max frequency intermediate syllable,  $Q_{50}$  intermediate syllable,  $F_0$  end intermediate syllable. (c, d) Histogram with the distribution of Kendall's tau with random acoustic data using 3000 Monte Carlo simulations for (c) males and (d) females. The vertical lines mark the Kendall's tau that we achieved with our real data for each sex.

for crested auklets, *Aethia cristatella*, sub adults (2-year-old birds) that show an increase in ornament size with age (Jones, Hunter, & Fraser, 2000). Underlying mechanisms of the production of ornamentation are preserved and similar across many species (Eliason, Maia, & Shawkey, 2015; Hill & McGraw, 2006b; Prum, Quinn, & Torres, 2006). Several bird species show delayed plumage maturation, that is, the 'delayed acquisition of a definitive colour and pattern of plumage until after the first potential breeding period in birds' (Hawkins, Hill, & Mercadante, 2012, p. 1). This has been

**Table 8**  
 $\beta$  coefficients, i.e. relative importance of the acoustic variables used in the ORI analysis that maximize Kendall's correlation between the linear index of these variables and adult age (one model per sex)

Sex	Variable	$\beta$
Male	Tempo	-0.811
	$F_{\text{Max}}$ first syllable	0.486
	Energy < 3000 Hz first syllable	0.329
Female	$Q_{50}$ intermediate syllable	0.835
	$F_{\text{Max}}$ intermediate syllable	0.487
	$F_0$ end intermediate syllable	-0.261

Variables are ranked from highest to lowest, with Tempo having the highest impact on the ranking for males and  $Q_{50}$  of the intermediate syllable for females.

shown in the northern flicker, *Colaptes auratus* (Wiebe & Vitousek, 2015), in adult male house finches, *Haemorhous mexicanus* (Inouye, Hill, Stradi, & Montgomerie, 2001), and European starlings, *Sturnus vulgaris* (Komdeur et al., 2005). Before the first foraging trip at sea, king penguins likely cannot acquire the resources to produce adult plumage (constrained moult hypothesis, Rohwer & Butcher, 1988). The only nutritional source for chicks is the predigested food they receive from their parents through regurgitation (Groscolas & Robin, 2001), while juveniles face considerable energetic challenges during their first foraging trips at sea (Enstipp et al., 2017, 2019). Chicks and juveniles might therefore be constrained in their energy investment in ornament production and might also gain benefits from being recognized as immature birds in a social context as explained by the delayed investment hypothesis (Hawkins et al., 2012) as their delayed plumage maturation signals a noncompetitive status to adults. They face less aggression during encounters with adults while being allowed to access the display area (H. J. Kriesell, personal observation).

The mean fundamental frequency of their calls decreased from chicks to juveniles and from juveniles to adults and differed significantly between males and females. We achieved high classification rates for male and female age classes based on only four

acoustic variables of fundamental frequency and energy quartile parameters. Mean fundamental frequency was not correlated with body size, and we lack evidence that a larger or smaller body size is advantageous for king penguins, for example in fending off predators or acquiring more resources. Furthermore, many studies have found contrasting results regarding the relationship between body weight or size and fundamental frequency of vocalizations (reviewed by Cardoso, 2012). Instead, it is likely that vocal tract characteristics, such as vibratory tissue size and tension, mineralization processes and muscle activity influence frequency parameters in birds (Düring et al., 2013; Goller & Riede, 2013; Larsen & Goller, 1999). Kriesell et al. (2020) investigated the vocal tract of king penguins and suggested that the two-voice phenomenon encoding individual identity in king penguins is a consequence of the bronchial syrinx type and its peculiar morphology. Although we need comparative anatomical studies to find support for this hypothesis specifically for king penguins, syrinx characteristics such as chondrification likely change with age and we consequently measure differences in vocal parameters depending on maturity.

Calls plus ornaments could offer a more accurate indication of age class than one signal modality alone and could increase the chances of a receiver responding (backup-up signal hypothesis; Johnstone, 1996; Møller & Pomiankowski, 1993). Individuals may also focus on different signals from the sender depending on the environment or the circumstances. In the crowded environment of king penguin colonies, vocalizations are effective over a relatively long distance (around 16 m; Aubin & Jouventin, 2002) when vision is impaired by conspecifics, and possibly in bad weather conditions, while visual signals, and probably chemical signals (East & Dehnhard, 2013; Gabriot, Buatois, Müller, & Bonadonna, 2018), are more efficient over short distances or when acoustic signals are masked by background noise (efficacy trade-off hypothesis; Hebets & Papaj, 2005). It might be advantageous for king penguins to perform a sequential analysis of signals, depending on the distance between receiver and sender (multiple sensory environment hypothesis; reviewed in Candolin, 2003). This has been shown for example in the Iberian wall lizard, *Podarcis hispanica*, which uses colour signals for long-distance sex recognition while odour is the more important signal for short-range communication (López & Martín, 2001).

We therefore need to consider that in the context of courtship display, call and ornament signals influence one another (inter-signal interaction hypotheses; reviewed in Hebets & Papaj, 2005). Part of this hypothesis is based on increased detection and discrimination by the receiver: producing a call could draw the attention of a receiver to the sender, thereby increasing the chances of the receiver detecting the sender's ornament signal. Another possibility is that king penguins are more likely to accurately memorize information about conspecifics if several sensory systems are targeted (receiver psychology hypothesis; Rowe, 1999). This might be advantageous for parading individuals, which are confronted with a rapidly changing social environment and numerous aggregations of individuals displaying courtship (Jouventin & Dobson, 2018; Keddar et al., 2013).

#### Calls Signal Age in Adults

Three sex-specific acoustic variables were sufficient to predict age for adult males and females using the ORI. Evidence exists that the production of brighter ornaments is energetically costly (eastern bluebirds, *Sialia sialis*, Siefferman et al., 2005; king penguins, Schull et al., 2017), and older birds might be facing a trade-off between investment in coloration and life-sustaining functions, such as immune function and oxidative stress balance (Kelly, Murphy, Tarvin, & Burness, 2012; Mougeot et al., 2010; Schull

et al., 2016). The lack of relationship between ornament parameters and age in king penguin females suggests that different evolutionary pressures act on the evolution of colourful ornaments for males and females (Viblanc et al., 2016), and that the evolutionary advantage to visually signal their age to potential partners might be stronger for males than for females. In the first phase of courtship display behaviour, king penguins form trios which consist of two males and one female, suggesting a male-biased contest (Keddar, Andirs, Bonadonna, & Dobson, 2013). Furthermore, male king penguins take over the first and longest shift of incubation on land (i.e. they have a longer fasting period), while females forage at sea after egg laying, and competition for territory is strong in king penguins (Keddar et al., 2015). Males may have to fight to acquire and subsequently defend the territory, especially during the first few weeks of settlement. An effect of male age, or more precisely experience, on successful territory acquisition and defence may thus have favoured the evolution of a signal of age in males, in the context of not just mate choice, but also male intra-sexual selection. King penguin ornament parameters have been shown to vary with rapidly changing physiological or environmental conditions (e.g. parasite load; Schull et al., 2016), oxidative status and acute stress (Viblanc et al., 2016) and immune system response (Schull et al., 2017). Thus, in this sexually monomorphic species, ornament parameters may be influenced by, and could therefore signal, rapidly changing or fluctuating physiological conditions but might not be a suitable signal of a constant individual trait like sex, or a gradually changing individual trait like age.

In contrast to ornaments, the relationship between acoustic parameters and age was strong in both sexes. We identified the tempo of calls as the most important factor linked to age in males, with a significant decrease with age. In two songbird species, the collared flycatcher, *Ficedula albicollis*, and the rock sparrow, *Petrotonia petronia*, male song rate was also shown to decrease with age (Garamszegi et al., 2007; Nemeth et al., 2012). Varilly and Chandler (2012) found a significant difference between middle-aged and old Bengalese finch, *Lonchura striata domestica*, males for whom song tempo decreased with age, due to longer intervals between syllables. The authors argued that the slower song rate may be caused by neurological changes. In king penguin females, the most important factor linked to adult age was the frequency below which 50% of the energy of an intermediate syllable was located, with older females producing syllables with a higher  $Q_{50}$  value. Spectral energy distribution may be connected to changes in the vibratory tissues, which has been shown by surgical removal of the medial tympaniform membranes in zebra finches, *Taeniopygia guttata* (Goller & Larsen 1997, 2002). The frequency of maximum amplitude of the first and middle syllables increased with age for males and females, respectively. Dominant frequencies of vocalizations may be modified through anatomical structures, that is, the oropharyngeal–oesophageal cavity (Hoese, Podos, Boetticher, & Nowicki, 2000; Riede & Suthers, 2009) or muscular activity (Goller & Suthers, 1996).

Our results indicate a maturation-related process and a progressive development of vocalizations. We hypothesize that calls have evolved to be an honest signal of adult age in the context of mate choice and suggest that the evolutionary advantage for king penguins to accurately signal their age acoustically to potential partners is relevant for both sexes. Schull et al. (2018) showed that king penguins paired assortatively according to telomere length, but not to body mass or size. Although controversial, there is evidence that telomere length is associated with age in birds (Hausmann & Heidinger, 2015; Heidinger et al., 2012). One plausible explanation therefore is that king penguins do pair assortatively according to age. Furthermore, long-term monitoring

programmes suggest that king penguins mate with partners of similar age (Le Bohec & Kriesell, n.d.).

In future studies, playback of calls recorded from individuals of different ages should be broadcast to courting individuals, ideally also of known age, to test the hypothesis that these vocalizations are a mate choice signal and one that conveys information about age to conspecifics. The playback of artificially modified signals to resemble callers of different ages could also provide information about the age preference of courting birds (Charlton & McComb, 2007).

#### *Ornaments and Calls Signal Sex and Breeding Status*

Parading male king penguin males displayed a higher UV beak saturation than parading females, and females showed a brighter beak spot. However, there was no sex difference in colour parameters between male and female breeders. Parading males and females had brighter and more saturated beak spots than breeding birds of the same sex. Sexual dimorphism has been studied extensively (Bonduriansky & Rowe, 2005; Coulter, 1986; Delestrade, 2001; Lande, 1980; Owens & Hartley, 1998; Ristow & Wink, 1980; Tschirren, Fitze, & Richner, 2003). The differences observed in this study are in line with the findings of Viblanc et al. (2016) who showed that the relations between certain physiological conditions and colour parameters were different or even opposite for the two sexes, suggesting that mutual mate choice can occur in king penguins but that different evolutionary pressures might act on the development of colourful ornaments in each sex.

Schull et al. (2016) showed that the UV brightness and YO chroma of the beak decreased during the fasting period of breeding birds. Furthermore, parasite load of breeding birds appeared to have a negative effect on the UV and YO coloration of their beak. The differences observed between parading and breeding birds could be explained by structural wearing out and physiological changes, for example related to fasting. This could apply to both sexes equally, which would explain why there were no differences between breeding males and females. However, these differences only concerned the beak, but not ear patch parameters. Ear patch coloration could serve functions other than mate choice in both sexes, for example social status and aggressiveness (Keddar et al., 2015; Viera, Nolan, Côté, Jouventin, & Groscolas, 2008).

In king penguins, sex differences in calls have been studied in adults (Kriesell et al., 2018). Here, we showed that the relation between syllable structure and sex is also valid for chicks and juveniles and can thus be used as a noninvasive tool for researchers in the field to determine the sex of study birds. The development of this sexual dimorphism may take place early during prehatching development and may rely on sex-specific hormonal regulation (reviewed by Gahr, 2007).

We showed that the calls of breeding birds are almost twice as long as those of parading birds ('short' and 'long' calls, Stonehouse, 1960). The signal context and environment differ between these two conditions. Producing more syllables, and thus more redundancy, might increase the chances of being identified by a specific individual at a distance in a noisy colony for breeding birds, while a parading bird might not target one specific individual. Common marmosets, *Callithrix jacchus*, have been shown to increase their call length in isolation, possibly to improve their chances of being localized by conspecifics (Norcross & Newman, 1993). The call duration in king penguins might therefore be shaped by environmental and contextual constraints (Lengagne, Aubin, Lauga, & Jouventin, 1999).

## CONCLUSIONS

To our knowledge, this is the first study to investigate ornaments and vocalizations simultaneously at the species level as bimodal signals of maturity and age in a seabird. In king penguins, age is an important piece of information when choosing a mate, because it is a predictor of breeding success and we showed that both ornamentation and acoustic parameters encoded age class and call parameters were correlated with adult age. Future research investigating different signal modalities and their interactions would allow us to draw new conclusions about the relative importance, and ultimately contribute to a better understanding, of the evolution of multimodal signalling.

## Data Availability

The data that support the plots within this paper and other findings of this study are available from the corresponding author upon request.

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## Supplementary Material

Supplementary material associated with this article can be found online at <https://doi.org/10.1016/j.anbehav.2021.05.015>.

## References

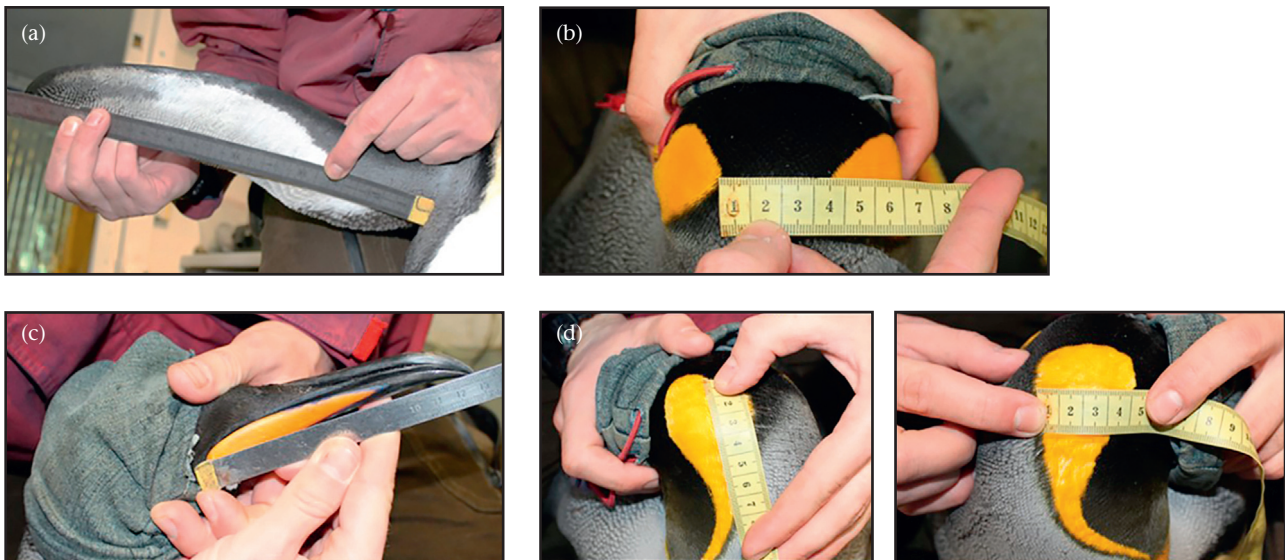
- Angelier, F., Moe, B., Weimerskirch, H., & Chastel, O. (2007). Age-specific reproductive success in a long-lived bird: Do older parents resist stress better? *Journal of Animal Ecology*, 76, 1181–1191. <https://doi.org/10.1111/j.1365-2656.2007.01295.x>
- Aubin, T., & Jouventin, P. (2002). How to vocally identify kin in a crowd: The penguin model. *Advances in the Study of Behavior*, 31, 243–277. [https://doi.org/10.1016/s0065-3454\(02\)80010-9](https://doi.org/10.1016/s0065-3454(02)80010-9)
- Aubin, T., Jouventin, P., & Hildebrand, C. (2000). Penguins use the two-voice system to recognize each other. *Proceedings of the Royal Society B: Biological Sciences*, 267, 1081–1087. <https://doi.org/10.1098/rspb.2000.1112>
- Barrat, A., Barré, H., Derenne, P., Fressangedu du Bost, D., Mary, G., Mougin, J.-L., et al. (1976). *Recherches écologiques sur les oiseaux de l'archipel Crozet*. Paris, France: Museum National d'Histoire Naturelle.
- Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: A practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society: Series B (Methodological)*, 57(1), 289–300. <https://doi.org/10.1111/j.2517-6161.1995.tb02031.x>
- Bonduriansky, R., & Rowe, L. (2005). Sexual selection, genetic architecture, and the condition dependence of body shape in the sexually dimorphic fly *Prochyliza xanthostoma* (Piophilidae). *Evolution*, 59(1), 138–151. <https://doi.org/10.1111/j.0014-3820.2005.tb00901.x>
- Boves, T. J., Buehler, D. A., Wood, P. B., Rodewald, A. D., Larkin, J. L., Keyser, P. D., et al. (2014). Multiple plumage traits convey information about age and within-age-class qualities of a canopy-dwelling songbird, the Cerulean Warbler. *The Auk: Ornithological Advances*, 131, 20–31. <https://doi.org/10.1642/auk-13-191.1>
- Bradley, J. S., Wooller, R. D., & Skira, I. J. (1995). The relationship of pair-bond formation and duration to reproductive success in short-tailed shearwaters *Puffinus tenuirostris*. *Journal of Animal Ecology*, 64(1), 31–38. <https://doi.org/10.2307/5825>
- Bro-Jørgensen, J. (2010). Dynamics of multiple signalling systems: Animal communication in a world in flux. *Trends in Ecology & Evolution*, 25(5), 292–300. <https://doi.org/10.1016/j.tree.2009.11.003>
- Budden, A. E., & Dickinson, J. L. (2009). Signals of quality and age: The information content of multiple plumage ornaments in male western bluebirds *Sialia*

- mexicana. *Journal of Avian Biology*, 40, 18–27. <https://doi.org/10.1111/j.1600-048X.2008.04344.x>
- Candolin, U. (2003). The use of multiple cues in mate choice. *Biological Reviews of the Cambridge Philosophical Society*, 78, 575–595. <https://doi.org/10.1017/S1464793103006158>
- Cardoso, G. C. (2012). Paradoxical calls: The opposite signaling role of sound frequency across bird species. *Behavioral Ecology*, 23(2), 237–241. <https://doi.org/10.1093/beheco/arr200>
- Catry, P., & Furness, R. W. (1999). The influence of adult age on territorial attendance by breeding great skuas *Catharacta skua*: An experimental study. *Journal of Avian Biology*, 30(4), 399–406. <https://doi.org/10.2307/3677012>
- Cézilly, F., Vincent, B., Tourenq, C. J., & Johnson, A. R. (1997). Age-assortative pairing in the greater flamingo *Phoenicopterus ruber roseus*. *Ibis*, 139(2), 331–336. <https://doi.org/10.1111/j.1474-919X.1997.tb04632.x>
- Charlton, B. D., Reby, D., & McComb, K. (2007). Female red deer prefer the roars of larger males. *Biology Letters*, 3(4), 382–385. <https://doi.org/10.1098/rsbl.2007.0244>
- Coulter, M. C. (1986). Assortative mating and sexual dimorphism in the Common tern. *Wilson Bulletin*, 98(1), 93–100. <https://doi.org/10.2307/4162187>
- De Kort, S. R., Eldermire, E. R. B., Valderrama, S., Botero, C. A., & Vehrencamp, S. L. (2009). Trill consistency is an age-related assessment signal in banded wrens. *Proceedings of the Royal Society B: Biological Sciences*, 276, 2315–2321. <https://doi.org/10.1098/rspb.2009.0127>
- De'Ath, G., & Fabricius, K. E. (2000). Classification and regression trees: A powerful yet simple technique for ecological data analysis. *Ecology*, 81(11), 3178–3192. [https://doi.org/10.1890/0012-9658\(2000\)081\[3178:CARTAP\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2000)081[3178:CARTAP]2.0.CO;2)
- Delestrade, A. (2001). Sexual size dimorphism and positive assortative mating in alpine choughs (*Pyrrhocorax graculus*). *The Auk: Ornithological Advances*, 118(2), 553–556.
- Delord, K., Barbraud, C., & Weimerskirch, H. (2004). Long-term trends in the population size of king penguins at Crozet archipelago: Environmental variability and density dependence? *Polar Biology*, 27, 793–800. <https://doi.org/10.1007/s00300-004-0651-z>
- Descamps, S., Gauthier-Clerc, M., Gendner, J.-P., & Maho, Y. Le (2002). The annual cycle of unbanded king penguins *Aptenodytes patagonicus* on Possession Island (Crozet). *Avian Science*, 2(2), 87–98.
- Dobson, F. S., Couchoux, C., & Jouventin, P. (2011). Sexual selection on a coloured ornament in king penguins. *Ethology*, 117, 872–879. <https://doi.org/10.1111/j.1439-0310.2011.01940.x>
- Dresp, B., Jouventin, P., & Langley, K. (2005). Ultraviolet reflecting photonic microstructures in the King Penguin beak. *Biology Letters*, 1, 310–313. <https://doi.org/10.1098/rsbl.2005.0322>
- Dubois, F., Cézilly, F., & Pagel, M. (1998). Mate fidelity and coloniality in waterbirds: A comparative analysis. *Oecologia*, 116(3), 433–440. <https://doi.org/10.1007/s004420050607>
- Düring, D. N., Ziegler, A., Thompson, C. K., Ziegler, A., Faber, C., Müller, J., et al. (2013). The songbird syrinx morphome: A three-dimensional, high-resolution, interactive morphological map of the zebra finch vocal organ. *BMC Biology*, 11(1). <https://doi.org/10.1186/1741-7007-11-1>
- East, M. L., & Dehnhard, M. (2013). *Chemical Signals in Vertebrates*. New York, NY: Springer. <https://doi.org/10.1007/9781461459279>
- Eliason, C. M., Maia, R., & Shawkey, M. D. (2015). Modular color evolution facilitated by a complex nanostructure in birds. *Evolution*, 69, 357–367. <https://doi.org/10.1111/evo.12575>
- Enstipp, M. R., Bost, C. A., Le Bohec, C., Bost, C., Laesser, R., Le Maho, Y., et al. (2019). The dive performance of immature king penguins following their annual molt suggests physiological constraints. *Journal of Experimental Biology*, 222, Article jeb208900. <https://doi.org/10.1242/jeb.208900>
- Enstipp, M. R., Bost, C. A., Le Bohec, C., Bost, C., Le Maho, Y., Weimerskirch, H., et al. (2017). Apparent changes in body insulation of juvenile king penguins suggest an energetic challenge during their early life at sea. *Journal of Experimental Biology*, 220, 2666–2678. <https://doi.org/10.1242/jeb.160143>
- Fahlman, A., et al. (2006). Accounting for body condition improves allometric estimates of resting metabolic rates in fasting king penguins, *Aptenodytes patagonicus*. *Polar Biology*, 29(7), 609–614. <https://doi.org/10.1007/s00300-005-0096-z>
- Favaro, L., Gamba, M., Gili, C., & Pessani, D. (2017). Acoustic correlates of body size and individual identity in banded penguins. *PLoS One*, 12(2), Article e0170001. <https://doi.org/10.1371/journal.pone.0170001>
- Gabirot, M., Buatois, B., Müller, C. T., & Bonadonna, F. (2018). Odour of King Penguin feathers analysed using direct thermal desorption discriminates between individuals but not sexes. *Ibis*, 160(2), 379–389. <https://doi.org/10.1111/ibi.12544>
- Gahr, M. (2007). Sexual differentiation of the vocal control system of birds. *Advances in Genetics*, 59(7), 67–105. [https://doi.org/10.1016/S0065-2660\(07\)59003-6](https://doi.org/10.1016/S0065-2660(07)59003-6)
- Garamszegi, L. Z., Török, J., Hegyi, G., Szöllösi, E., Rosivall, B., & Eens, M. (2007). Age-dependent expression of song in the collared flycatcher, *Ficedula albicollis*. *Ethology*, 113(3), 246–256. <https://doi.org/10.1111/j.1439-0310.2007.01337.x>
- Gauthier-Clerc, M., Le Maho, Y., Gendner, J. P., Durant, J., & Handrich, Y. (2001). State-dependent decisions in long-term fasting King Penguins, *Aptenodytes patagonicus*, during courtship and incubation. *Animal Behaviour*, 62, 661–669. <https://doi.org/10.1006/anbe.2001.1803>
- Gendner, J.-P., Gauthier-Clerc, M., Le Bohec, C., Descamps, S., & Le Maho, Y. (2005). A new application for transponders in studying penguins. *Journal of Field Ornithology*, 76(2), 138–142. <https://doi.org/10.1648/0273-8570-76.2.138>
- Goller, F., & Larsen, O. N. (1997). In situ biomechanics of the syrinx and sound generation in pigeons. *Journal of Experimental Biology*, 200(Pt 16), 2165–2176.
- Goller, F., & Larsen, O. N. (2002). New perspectives on mechanisms of sound generation in songbirds. *Journal of Comparative Physiology: Neuroethology, Sensory, Neural, and Behavioral Physiology*, 188(11–12), 41–50. <https://doi.org/10.1007/s00359-002-0350-6>
- Goller, F., & Riede, T. (2013). Integrative physiology of fundamental frequency control in birds. *Journal of Physiology*, 107, 230–242. <https://doi.org/10.1016/j.jphysparis.2012.11.001>
- Goller, F., & Suthers, R. A. (1996). Role of syrinxal muscles in gating airflow and sound production in singing brown thrashers. *Journal of Neurophysiology*, 75(2), 867–876. <https://doi.org/10.1152/jn.1996.75.2.867>
- Groscolas, R., & Cherel, Y. (2007). How to molt while fasting in the cold: The metabolic and hormonal adaptations of emperor and king penguins. *Ornis Scandinavica*, 23(3), 328–334. <https://doi.org/10.2307/3676657>
- Groscolas, R., Decrock, F., Thil, M. A., Fayolle, C., Boissery, C., & Robin, J. P. (2000). Refeeding signal in fasting-incubating king penguins: Changes in behavior and egg temperature. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology*, 279, 2104–2112. <https://doi.org/10.1152/ajpregu.2000.279.6.r2104>
- Groscolas, R., & Robin, J. P. (2001). Long-term fasting and re-feeding in penguins. *Comparative Biochemistry and Physiology*, 128(3), 645–655. [https://doi.org/10.1016/S1096-4933\(00\)00341-X](https://doi.org/10.1016/S1096-4933(00)00341-X)
- Hatch, S. A. (1990). Individual variation in breeding and hatching success of Northern fulmars. *The Auk: Ornithological Advances*, 107(4), 750–755.
- Hausmann, M. F., & Heidinger, B. J. (2015). Telomere dynamics may link stress exposure and ageing across generations. *Biology Letters*, 11(11), 20150396. <https://doi.org/10.1098/rsbl.2015.0396>
- Hawkins, G. L., Hill, G. E., & Mercadante, A. (2012). Delayed plumage maturation and delayed reproductive investment in birds. *Biological Reviews*, 87(2), 257–274. <https://doi.org/10.1111/j.1469-185X.2011.00193.x>
- Hayes, T., Usami, S., Jacobucci, R., & Mcardle, J. J. (2015). Using classification and regression trees (CART) and random forests to analyze attrition: Results from two simulations. *Psychology and Aging*, 30(4), Article pag0000046. <https://doi.org/10.1037/pag0000046.supp>
- Hebets, E. A., & Papaj, D. R. (2005). Complex signal function: Developing a framework of testable hypotheses. *Behavioral Ecology and Sociobiology*, 57, 197–214. <https://doi.org/10.1007/s00265-004-0865-7>
- Heidinger, B. J., Blount, J. D., Boner, W., Griffiths, K., Metcalfe, N. B., & Monaghan, P. (2012). Telomere length in early life predicts life span. *Proceedings of the National Academy of Sciences of the United States of America*, 109(5), 1743–1748. <https://doi.org/10.1289/EHP518>
- Higham, J. P., & Hebets, E. A. (2013). An introduction to multimodal communication. *Behavioral Ecology and Sociobiology*, 67(9), 1381–1388. <https://doi.org/10.1007/s00265-013-1590-x>
- Hill, G. E., & McGraw, K. J. (2006a). *Bird Coloration: Mechanisms and Measurements*. Cambridge, MA: Harvard University Press.
- Hill, G., & McGraw, K. (2006b). *Bird Coloration: Function and Evolution*. Cambridge, Massachusetts: Harvard University Press.
- Hoes, W. J., Podos, J., Boetticher, N. C., & Nowicki, S. (2000). Vocal tract function in birdsong production: Experimental manipulation of beak movements. *Journal of Experimental Biology*, 203(12), 1845–1855. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/10821742>
- Inouye, C. Y., Hill, G. E., Stradi, R. D., & Montgomerie, R. (2001). Carotenoid pigments in male house finch plumage in relation to age, subspecies, and ornamental coloration. *The Auk: Ornithological Advances*, 118(4), 900–915. [https://doi.org/10.1642/0004-8038\(2001\)118\[0900:CPIMHF\]2.0.CO;2](https://doi.org/10.1642/0004-8038(2001)118[0900:CPIMHF]2.0.CO;2), 10.1642/0004-8038%282001%29118%5B0900%3ACPIMHF
- Johnstone, R. A. (1996). Multiple displays in animal communication: 'Backup signals' and 'multiple messages'. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 351, 329–338. <https://doi.org/10.1098/rstb.1996.0026>
- Jones, I. L., Hunter, F. M., & Fraser, G. (2000). Patterns of variation in ornaments of crested auklets *Aethia cristatella*. *Journal of Avian Biology*, 2, 119–127.
- Jouventin, P. (1982). *Visual and vocal signals in penguins, their evolution and adaptive characters*. Berlin, Germany: Paul Parey.
- Jouventin, P., Aubin, T., & Lengagne, T. (1999b). Finding a parent in a king penguin colony: The acoustic system of individual recognition. *Animal Behaviour*, 57(6), 1175–1183. <https://doi.org/10.1163/156853999501595>
- Jouventin, P., & Dobson, F. S. (2018). *Why Penguins Communicate. The Evolution of Visual and Vocal Signals*. Amsterdam, Netherlands: Elsevier Academic Press.
- Jouventin, P., Lequette, B., & Dobson, F. (1999a). Age-related mate choice in the wandering albatross. *Animal Behaviour*, 57(5), 1099–1106. <https://doi.org/10.1006/anbe.1999.1083>
- Jouventin, P., Nolan, P. M., Dobson, F. S., & Nicolaus, M. (2008). Coloured patches influence pairing rate in King Penguins. *Ibis*, 150, 193–196. <https://doi.org/10.1111/j.1474-919X.2007.00749.x>
- Jouventin, P., Nolan, P. M., Örnborg, J., & Dobson, F. S. (2005). Ultraviolet beak spots in king and emperor penguins. *The Condor: Ornithological Applications*, 107, 144–150. <https://doi.org/10.1650/7512>

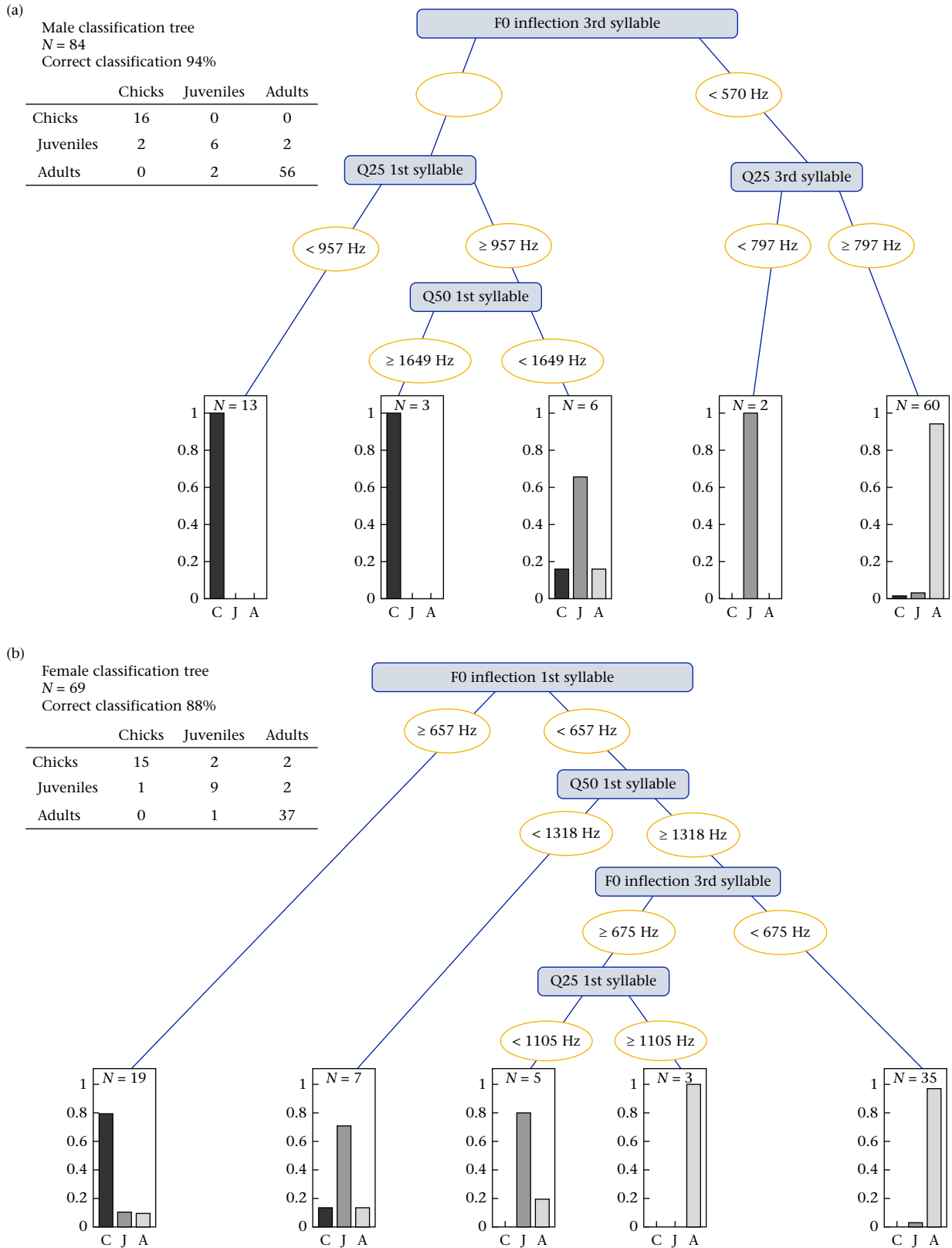
- Keddar, I., Altmeyer, S., Couchoux, C., Jouventin, P., & Dobson, F. S. (2015a). Mate choice and colored beak spots of king penguins. *Ethology*, *121*, 1048–1058. <https://doi.org/10.1111/eth.12419>
- Keddar, I., Andris, M., Bonadonna, F., & Dobson, F. S. (2013). Male-biased mate competition in king penguin trio parades. *Ethology*, *119*(5), 389–396. <https://doi.org/10.1111/eth.12076>
- Keddar, I., Jouventin, P., & Dobson, F. S. (2015b). Color ornaments and territory position in king penguins. *Behavioural Processes*, *119*, 32–37. <https://doi.org/10.1016/j.beproc.2015.07.003>
- Kelly, R. J., Murphy, T. G., Tarvin, K. A., & Burness, G. (2012). Carotenoid-based ornaments of female and male American goldfinches (*Spinus tristis*) show sex-specific correlations with immune function and metabolic rate. *Physiological and Biochemical Zoology*, *85*(4), 348–363. <https://doi.org/10.1086/666059>
- Komdeur, J., Oorebeek, M., Van Overveld, T., & Cuthill, I. C. (2005). Mutual ornamentation, age, and reproductive performance in the European starling. *Behavioral Ecology*, *16*(4), 805–817. <https://doi.org/10.1093/beheco/ari059>
- Kraaijeveld, K. (2003). Degree of mutual ornamentation in birds is related to divorce rate. *Proceedings of the Royal Society B: Biological Sciences*, *270*(1526), 1785–1791. <https://doi.org/10.1098/rspb.2003.2450>
- Kriesell, H. J., Aubin, T., Planas-Bielsa, V., Benoiste, M., Bonadonna, F., Gachot-Neveu, H., et al. (2018). Sex identification in King Penguins *Aptenodytes patagonicus* through morphological and acoustic cues. *Ibis*, *160*, 755–768. <https://doi.org/10.1111/ibi.12577>
- Kriesell, H. J., Le Bohec, C., Cerwenka, A. F., Robin, J.-P., Ruthensteiner, B., Gahr, M., et al. (2020). Vocal tract anatomy of king penguins: Morphological traits of two-voiced sound production. *Frontiers in Zoology*, *17*(5). <https://doi.org/10.1186/s12983-020-0351-8>
- Lande, R. (1980). Sexual dimorphism, sexual selection, and adaptation in polygenic characters. *Evolution*, *34*(2), 292. <https://doi.org/10.2307/2407393>
- Larsen, O. N., & Goller, F. (1999). Role of syrinx vibrations in bird vocalizations. *Proceedings of the Royal Society B: Biological Sciences*, *266*(1429), 1609–1615. <https://doi.org/10.1098/rspb.1999.0822>
- Le Bohec, C. (2007). *Stratégies d'histoire de vie d'un oiseau longévif: le manchot royal (Aptenodytes patagonicus)*. PhD thesis. Strasbourg, France: Université de Strasbourg.
- Le Bohec, C., & Kriesell, H. J. (n.d.). [Assortative mating according to age in king penguins]. (Unpublished raw data).
- Le Vaillant, M., Le Bohec, C., Prud'Homme, O., Wienecke, B., Le Maho, Y., Kato, A., et al. (2013). How age and sex drive the foraging behaviour in the king penguin. *Marine Biology*, *160*, 1147–1156. <https://doi.org/10.1007/s00227-013-2167-y>
- Le Vaillant, M., Wilson, R. P., Kato, A., Saraux, C., Hanuise, N., Prud'Homme, O., et al. (2012). King penguins adjust their diving behaviour with age. *Journal of Experimental Biology*, *215*(21), 3685–3692. <https://doi.org/10.1556/MPszle.67.2012.2.3>
- Lengagne, T., Aubin, T., Lauga, J., & Jouventin, P. (1999). How do king penguins (*Aptenodytes patagonicus*) apply the mathematical theory of information to communicate in windy conditions? *Proceedings of the Royal Society B: Biological Sciences*, *266*, 1623–1628. <https://doi.org/10.1098/rspb.1999.0824>
- Lengagne, T., Lauga, J., & Aubin, T. (2001). Intra-syllabic acoustic signatures used by the king penguin in parent-chick recognition: An experimental approach. *Journal of Experimental Biology*, *204*, 663–672. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/11171348>.
- López, P., & Martín, J. (2001). Pheromonal recognition of females takes precedence over the chromatic cue in male Iberian wall lizards *Podarcis hispanica*. *Ethology*, *107*(10), 901–912. <https://doi.org/10.1046/j.1439-0310.2001.00724.x>
- Madsen, V., Balsby, T. J. S., Dabelsteen, T., & Osorno, J. L. (2004). Bimodal signaling of a sexually selected trait: Gular pouch drumming in the magnificent frigatebird. *The Condor: Ornithological Applications*, *106*(1), 156–160. <https://doi.org/10.1650/7315>
- Madsen, V., Dabelsteen, T., Osorio, D., & Osorno, J. L. (2007). Morphology and ornamentation in male magnificent frigatebirds: Variation with age class and mating status. *The American Naturalist*, *169*, 93–111. <https://doi.org/10.1038/bjc.1997.262>
- Massaro, M., Davis, L. S., & Darby, J. T. (2003). Carotenoid-derived ornaments reflect parental quality in male and female yellow-eyed penguins (*Megadyptes antiopodes*). *Behavioral Ecology and Sociobiology*, *55*(2), 169–175. <https://doi.org/10.1007/s00265-003-0683-3>
- McGraw, K. J., Toomey, M. B., Nolan, P. M., Morehouse, N. I., Massaro, M., & Jouventin, P. (2007). A description of unique fluorescent yellow pigments in penguin feathers. *Pigment Cell Research*, *20*, 301–304. <https://doi.org/10.1111/j.1600-0749.2007.00386.x>
- McGraw, K. J., Wakamatsu, K., Ito, S., Nolan, P. M., Jouventin, P., Dobson, F. S., et al. (2004). You can't judge a pigment by its color: Carotenoid and melanin content of yellow and Brown feathers in swallows, bluebirds, penguins, and domestic chickens. *The Condor: Ornithological Applications*, *106*, 390–395. <https://doi.org/10.1650/7384>
- Møller, A. P., & Pomiankowski, A. (1993). Why have birds got multiple sexual ornaments? *Behavioral Ecology and Sociobiology*, *32*(3), 167–176. <https://doi.org/10.1007/BF00173774>
- Montgomerie, R. (2006). Analyzing colors. In G. E. Hill, & K. J. McGraw (Eds.), *Bird Coloration* (pp. 90–147). Cambridge, MA: Harvard University Press.
- Mougeot, F., Martínez-Padilla, J., Blount, J. D., Pérez-Rodríguez, L., Webster, L. M. I., & Pieltney, S. B. (2010). Oxidative stress and the effect of parasites on a carotenoid-based ornament. *Journal of Experimental Biology*, *213*, 400–407. <https://doi.org/10.1111/j.1420-9101.2010.02045.x>
- Nakagawa, S., & Schielzeth, H. (2010). Repeatability for Gaussian and non-Gaussian data: A practical guide for biologists. *Biological Reviews*, *85*(4), 935–956. <https://doi.org/10.1111/j.1469-185X.2010.00141.x>
- Nemeth, E., Kempenaers, B., Matessi, G., & Brumm, H. (2012). Rock sparrow song reflects male age and reproductive success. *PLoS One*, *7*(8), Article e43259. <https://doi.org/10.1371/journal.pone.0043259>
- Nicolaus, M., Le Bohec, C., Nolan, P. M., Gauthier-Clerc, M., Le Maho, Y., Komdeur, J., et al. (2007). Ornamental colors reveal age in the king penguin. *Polar Biology*, *31*, 53–61. <https://doi.org/10.1007/s00300-007-0332-9>
- Nisbet, I. C. T., Winchell, J. M., & Heise, A. E. (1984). Influence of age on the breeding biology of common terns. *Colonial Waterbirds*, *7*, 117–126. <https://doi.org/10.2307/1521090>
- Nolan, P. M., Dobson, S. F., Nicolaus, M., Karels, T. J., McGraw, K. J., & Jouventin, P. (2010). Mutual mate choice for colorful traits in king penguins. *Ethology*, *116*, 635–644. <https://doi.org/10.1111/j.1439-0310.2010.01775.x>
- Norcross, J. L., & Newman, J. D. (1993). Context and gender-specific differences in the acoustic structure of common marmoset (*Callithrix jacchus*) phoe calls. *American Journal of Primatology*, *30*(1), 37–54. <https://doi.org/10.1002/ajp.1350300104>
- Olsson, O. (1997). Clutch abandonment: A state-dependent decision in king penguins. *Journal of Avian Biology*, *28*(3), 264–266.
- Owens, I. P. F., & Hartley, I. R. (1998). Sexual dimorphism in birds: Why are there so many different forms of dimorphism? *Proceedings of the Royal Society B: Biological Sciences*, *265*, 397–407. <https://doi.org/10.1098/rspb.1998.0308>
- Partan, S. R. (2013). Ten unanswered questions in multimodal communication. *Behavioral Ecology and Sociobiology*, *67*(9), 1523–1539. <https://doi.org/10.1007/s00265-013-1565-y>
- Pince, G., Dobson, F. S., & Jouventin, P. (2009). Experiments on colour ornaments and mate choice in king penguins. *Animal Behaviour*, *78*, 1247–1253. <https://doi.org/10.1016/j.anbehav.2009.07.041>
- Planas-Bielsa, V., Ortega J.-P., Eckbo, N. H., & Le Bohec, C. (n.d.). Optimal response index and its applications in ecology. (Manuscript in preparation).
- Prum, R. O., Quinn, T., & Torres, R. H. (2006). Anatomically diverse butterfly scales all produce structural colours by coherent scattering. *Journal of Experimental Biology*, *209*, 748–765. <https://doi.org/10.1242/jeb.02051>
- R Core Team. (2013). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.
- Riede, T., & Suthers, R. A. (2009). Vocal tract motor patterns and resonance during constant frequency song: The white-throated sparrow. *Journal of Comparative Physiology: Neuroethology, Sensory, Neural, and Behavioral Physiology*, *195*(2), 183–192. <https://doi.org/10.1007/s00359-008-0397-0>
- Ristow, D., & Wink, M. (1980). Sexual dimorphism of cory's shearwater. *II Meril*, *21*, 9–12.
- Rivera-Gutierrez, H. F., Pinxten, R., & Eens, M. (2010). Multiple signals for multiple messages: Great tit, *Parus major*, song signals age and survival. *Animal Behaviour*, *80*, 451–459. <https://doi.org/10.1016/j.anbehav.2010.06.002>
- Rohwer, S., & Butcher, G. S. (1988). Winter versus summer explanations of delayed plumage maturation in temperate passerine. *Science*, *131*(4), 556–572. <https://doi.org/10.1126/science.113.2924.43>
- Rosenthal, M. F., Wilkins, M. R., Shizuka, D., & Hebets, E. A. (2018). Dynamic changes in display architecture and function across environments revealed by a systems approach to animal communication. *Evolution*, *72*(5), 1134–1145. <https://doi.org/10.1111/evo.13448>
- Rowe, C. (1999). Receiver psychology and the evolution of multicomponent signals. *Animal Behaviour*, *58*, 921–931. <https://doi.org/10.1006/anbe.1999.1242>
- Saraux, C., Viblanc, V. A., Hanuise, N., Le Maho, Y., & Bohec, C. (2011). Effects of individual pre-fledging traits and environmental conditions on return patterns in Juvenile King Penguins. *PLoS One*, *6*(6), Article e20407. <https://doi.org/10.1371/journal.pone.0020407>
- Schull, Q., Dobson, F. S., Stier, A., Robin, J.-P., Bize, P., & Viblanc, V. A. (2016). Beak color dynamically signals changes in fasting status and parasite loads in king penguins. *Behavioral Ecology*, *27*(6), 1684–1693. <https://doi.org/10.1093/beheco/arw091>
- Schull, Q., Robin, J. P., Dobson, F. S., Saadaoui, H., Viblanc, V. A., & Bize, P. (2017). Experimental stress during molt suggests the evolution of condition-dependent and condition-independent ornaments in the king penguin. *Ecology and Evolution*, *8*(2), 1084–1095. <https://doi.org/10.1002/ece3.3677>
- Schull, Q., Viblanc, V. A., Dobson, F. S., Robin, J. P., Zahn, S., Cristofari, R., et al. (2018). Assortative pairing by telomere length in King Penguins (*Aptenodytes patagonicus*) and relationships with breeding success. *Canadian Journal of Zoology*, *96*(6), 639–647. <https://doi.org/10.1139/cjz-2017-0094>
- Siefferman, L., Hill, G. E., & Dobson, F. S. (2005). Ornamental plumage coloration and condition are dependent on age in eastern bluebirds *Sialia sialis*. *Journal of Avian Biology*, *36*(5), 428–435. <https://doi.org/10.1111/j.0908-8857.2005.03401.x>
- Song, Y. Y., & Lu, Y. (2015). Decision tree methods: Applications for classification and prediction. *Shanghai Archives of Psychiatry*, *27*(2), 130–135. <https://doi.org/10.11919/j.issn.1002-0829.215044>
- Stonehouse, B. (1960). Falkland Islands Dependencies Survey Scientific Reports. *The king penguin Aptenodytes patagonica of South Georgia island. Breeding behaviour and development* (Vol. 23). London, U.K.: Her Majesty's Stationery Office.
- Toscani, C. (2010). Le divorce chez le Manchot royal *Aptenodytes patagonicus*: un compromis entre synchronie des arrivées et choix du partenaire. Université de Strasbourg.

- Tschirren, B., Fitze, P. S., & Richner, H. (2003). Sexual dimorphism in susceptibility to parasites and cell-mediated immunity in great tit nestlings. *Journal of Animal Ecology*, 72(5), 839–845. <https://doi.org/10.1046/j.1365-2656.2003.00755.x>
- Varilly, P., & Chandler, D. (2012). The mixed block/event-related design. *NeuroImage*, 2, 265–275. <https://doi.org/10.1007/s10955-011-0269-9>
- Viblanç, V. A., Dobson, F. S., Stier, A., Schull, Q., Sarau, C., Gineste, B., et al. (2016). Mutually honest? Physiological 'qualities' signalled by colour ornaments in monomorphic king penguins. *Biological Journal of the Linnean Society*, 118, 200–214. <https://doi.org/10.1111/bj.12729>
- Viblanç, V. A., Smith, A. D., Gineste, B., & Groscolas, R. (2012). Coping with continuous human disturbance in the wild: Insights from penguin heart rate response to various stressors. *BMC Ecology*, 12(10). <https://doi.org/10.1186/1472-6785-12-10>
- Viera, V. M., Nolan, P. M., Côté, S. D., Jouventin, P., & Groscolas, R. (2008). Is territory defence related to plumage ornaments in the King penguin *Aptenodytes patagonicus*? *Ethology*, 114(2), 146–153. <https://doi.org/10.1111/j.1439-0310.2007.01454.x>
- Weimerskirch, H. (1992). Reproductive effort in long-lived birds: Age-specific patterns of condition, reproduction and survival in the wandering albatross. *Oikos*, 64(3), 464–473. <https://doi.org/10.2307/3545162>
- Weimerskirch, H., Stahl, J. C., & Jouventin, P. (1992). The breeding biology and population dynamics of King Penguins *Aptenodytes patagonica* on the Crozet Islands. *Ibis*, 134, 107–117. <https://doi.org/10.1111/j.1474-919X.1992.tb08387.x>
- Wiebe, K. L., & Vitousek, M. N. (2015). Melanin plumage ornaments in both sexes of Northern Flicker are associated with body condition and predict reproductive output independent of age. *The Auk: Ornithological Advances*, 132(2), 507–517. <https://doi.org/10.1642/auk-14-281.1>
- Zimmer, I., Ropert-Coudert, Y., Kato, A., Ancel, A., & Chiaradia, A. (2011). Does foraging performance change with age in female little penguins (*Eudyptula minor*)? *PLoS One*, 6(1), Article e16098. <https://doi.org/10.1371/journal.pone.0016098>
- Zipple, M. N., Nowicki, S., Searcy, W. A., & Peters, S. (2019). Full life course analysis of birdsong reveals maturation and senescence of highly repeatable song characteristics. *Behavioral Ecology*, 30(6), 1761–1768. <https://doi.org/10.1093/beheco/arz146>
- Zipple, M. N., Peters, S., Searcy, W. A., & Nowicki, S. (2020). Sounds of senescence: Male swamp sparrows respond less aggressively to the songs of older individuals. *Behavioral Ecology*, 31(2), 533–539. <https://doi.org/10.1093/beheco/arz218>

## Appendix



**Figure A1.** The lengths of (a) the flipper and (b) the beak were used in a principal component analysis to calculate a structural size index (SSI). (c) Apical distance was measured as the smallest distance between the yellow patches on top of the head. (d) Ear patch size was measured as the vertical and in a perpendicular line the horizontal length of the ear patches. We subsequently calculated the ear patch size as an ellipse surface.



**Figure A2.** Classification tree of acoustic parameters for age classes (C = chicks, J = juveniles, a = Adults) for (a) male and (b) female king penguins. The algorithm selected four variables per sex to classify individuals into age categories. Six individuals were misclassified in the male tree and eight in the female tree. Tables show the confusion matrix of predicted classes (rows) versus actual classes (columns). All correct predictions make up the diagonal of the table. The factors are classified into a hierarchy of branches. Points of ramifications of the branches are called 'nodes'. The first node ('root node', for females F0 inflection of first syllable and for males F0 inflection of intermediate syllable) represents the variable that splits the data into subnodes. Since the acoustic variables in our model were all continuous, the splitting values are shown as 'less than' and 'greater or equal to' the splitting value of the respective node variable. Note that the variables used in the trees are not rescaled. This had no impact on the outcome of the tree model but allows for a better interpretability of the tree. When terminal nodes are reached, the resulting classification in the age categories of individuals based on acoustic parameters is given in histograms, where N is the number of observations in each terminal node; these add up to the total number of observations ( $N_{\text{males}} = 84$ ,  $N_{\text{females}} = 69$ ).